EXHIBIT A - Additional Claim Amendments
(deletions indicated by strikethrough and additions underlined)

The previously described claims were additionally amended as follows:

24. A method of preparing a creatine amidinohydrolase comprising:

(i) mutating (a) the nucleic acid sequence of SEQ ID NO: 2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 1 to provide mutant nucleic acid sequences,

(ii) determining Km values <u>for creatine</u> of proteins encoded by the mutant nucleic acid sequences in a coupling assay using a sarcosine oxidase and a peroxidase,

(iii) selecting and isolating a desired mutant nucleic acid sequence that encodes a creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + $H_2O \rightarrow$ sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM,

<u>Molecular weight: about 43,000 (SDS-PAGE)</u>

<u>Isoelectic point: about 4.5</u>

<u>Optimum temperature: about 40-50 °C (at pH of about 7.5)</u>

<u>Optimum pH: about 8.0-9.0 (at a temperature of about 37 °C)</u>

(iv) expressing the desired mutant nucleic acid sequence in a host to produce creatine amidinohydrolase, and

(v) harvesting the produced creatine amidinohydrolase.

Cancel claims 25.-28.

Add claims 29-30.

29. <u>The method of claim 24, wherein the sarcosine oxidase is originated from the genus Arthrobacter, Corynebacterium, Alcaligenes, Pseudomonas, Micrococcus, or Bacillus.</u>

30. A method of preparing a creatine amidinohydrolase comprising:

(i) selecting (a) a nucleic acid sequence of SEQ ID NO: 2 or (b) a nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 1 to provide a source nucleic acid sequence,

(ii) mutating the source nucleic acid sequence to provide mutant nucleic acid sequences that encode mutant creatine amidinohydrolases,

(iii) selecting a mutant nucleic acid sequence that encodes a creatine amidinohydrolase which has a reduced Km value as compared to the Km value of creatine amidinohydrolase encoded by the source nucleic acid sequence by:

(A) determining a first activity of creatine amidinohydrolase encoded by the source nucleic acid sequence with a first concentration of a substrate and a second activity of creatine amidinohydrolase encoded by the source nucleic acid sequence with a second concentration of the substrate, wherein the second concentration of the substrate is less than the first concentration of the substrate,

(B) determining a first activity of the mutant creatine amidinohydrolase with the first concentration of the substrate and a second activity of the mutant creatine amidinohydrolase with the second concentration of the substrate, wherein the second concentration of the substrate is less than the first concentration of the substrate,

(C) calculating a ratio of the second activity of the creatine amidinohydrolase encoded by the source nucleic acid sequence divided by the first activity of the creatine amidinohydrolase encoded by the source nucleic acid sequence,

(D) calculating a ratio of the second activity of the mutant creatine amidinohydrolase divided by the first activity of the mutant creatine amidinohydrolase,

(E) comparing the ratio calculated in step (iii)(C) to the ratio calculated in step (iii)(D), wherein a mutant creatine amidinohydrolase that has a reduced Km value as compared to the Km value of creatine amidinohydrolase encoded by the source nucleic acid sequence has a greater ratio than the ratio for creatine amidinohydrolate encoded by the source nucleic acid,

(iv) selecting and isolating a desired mutant nucleic acid sequence that encodes a creatine amidinohydrolase having the following physicochemical properties:

Action: catalyzing the following reaction:

creatine + H_2O → sarcosine + urea

Km values for creatine in a coupling assay using a sarcosine oxidase and a peroxidase: 3.5-10.0 mM,

Molecular weight: about 43,000 (SDS-PAGE)

Isoelectic point: about 4.5

Optimum temperature: about 40-50 °C (at pH of about 7.5)

Optimum pH: about 8.0-9.0 (at a temperature of about 37 °C)

(v) expressing the desired mutant nucleic acid sequence in a host to produce creatine amidinohydrolase, and

(vi) harvesting the produced creatine amidinohydrolase.